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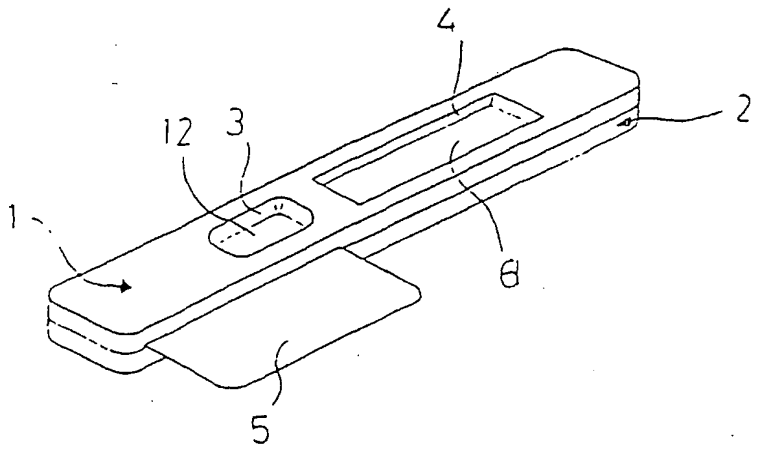
(54) Title: MULTI-ANALYTE ASSAY DEVICE WITH MULTI-SPOT DETECTION ZONE



multi-analyte and/or multi-specificity detection.

(57) Abstract: The present invention relates to a solid phase assay device comprising a multi-spot detection zone, and to use thereof in immunochromatographic assays. More precisely, the invention relates to a device for determining analytes in an aqueous sample comprising: an elongate flow matrix (6) allowing lateral transport of fluid therethrough, wherein said matrix comprises a sample application zone (3) and downstream thereof, a detection zone (8) having immobilised capture agents capable of directly or indirectly binding to said analytes, wherein said analytes are detected by allowing a labelled second binding agent to bind directly or indirectly to the analytes. The device is characterised in that the immobilised capture agents are distributed in the detection zone (8) as a plurality of small spots, thereby permitting

WO 03/025573 A1

MULTI-ANALYTE ASSAY DEVICE WITH MULTI-SPOT DETECTION ZONE

Field of the invention

The present invention relates to a solid phase assay device comprising a multi-spot detection zone, and to use thereof in immunochromatographic assays.

Background of the invention

A type of solid phase assay devices comprises a plate-shaped flow matrix of bibulous material, usually a membrane strip, such as of cellulose nitrate or glass fibre, in which liquid can be transported laterally (i.e. in the plane of the strip) by capillary forces in the membrane. The membrane usually has a sample application zone and a detection zone downstream of the sample application zone. In the detection zone, usually a capturing reagent for the analyte is immobilised. To conduct an assay, the application zone is contacted with the liquid sample to be assayed for the analyte of interest. The device is maintained under conditions sufficient to allow capillary action of liquid to transport the analyte of interest, if present in the sample, through the membrane strip to the detection zone where the analyte is captured. An absorbing pad or the like at the downstream end of the strip usually insures the capillary liquid flow. A detection reagent, usually labelled, is then added upstream of the detection zone and interacts with captured analyte in the detection zone, and the amount of captured analyte is measured. Often, the detection reagent is pre-disposed in or on the membrane strip, e.g. in the form of diffusely movable particles containing fluorophoric or chromogenic groups, either upstream of the sample application zone or between sample application zone and the detection zone.

A major drawback with these known devices is that only a few analytes can be measured per assay.

In EP 191 640 (Syntex Inc) there is disclosed a device in which more than one analyte may be detected. However, the number of analytes that may be detected is limited and the problem of detecting cross-reacting analytes is not addressed.

Summary of the invention

The problem underlying the present invention was to enable detection of several analytes and even analytes which cross-react with each other, such as different allergens reacting with the same IgE's.

This problem has been solved by a multi-spot device according to the present invention.

Thus, in a first aspect the invention provides a device for determining analytes in an aqueous sample comprising:
an elongate flow matrix allowing lateral transport of fluid therethrough, wherein said matrix comprises a sample application zone and downstream thereof, a detection zone having immobilised capture agents capable of directly or indirectly binding to said analytes, wherein said analytes are detected by allowing a labelled second binding agent to bind directly or indirectly to the analytes, **characterised in** that A) the immobilised capture agents are distributed in the detection zone as a plurality of small spots, thereby permitting multi-analyte and/or multi-specificity detection, and B) the capture agents are anchored to the matrix via immobilised particles, and C) the number of spots per flow matrix is more than 10, and D) wherein some of the spots functions as positive control(s) and/or internal calibrator(s).

The number of spots per flow matrix is preferably 5-1000, and more preferably 10-100. The spots are preferably smaller than 1 mm in diameter, preferably smaller than 0.5 mm in diameter.

The spots are preferably arranged in a pattern that allows for detection of cross reactive analytes or specificities. This is exemplified by allergens having cross-reacting IgE, i.e. such allergens should not be arranged in the same flow line.

The flow matrix may be a porous membrane, such as nitro-cellulose or a strip of solid material.

The capture agents may be antibodies or an immunoactive fragment thereof.
Alternatively, the capture agents are allergens or an immunoactive fragment thereof.

In another alternative, the capture agents are DNA/RNA, preferably single stranded or aptameres.

In a preferred embodiment of the device some of the spots functions as positive control(s) and/or internal calibrator(s).

The sample is whole blood, serum, plasma, saliva or urine.
The label of the labelled second binding reagent is, for example a fluorophore or a chromophore.

The device may be used for screening of unknown specificities as well as for detection of specific immunoglobulins. By depositing many spots with known material, for example protein or DNA etc, it is possible to rapidly screen for which binder(s) there are in a sample that are specifically binding to the material in particular spot(s). An example is sample determination of specific IgE, wherein the spots contain different allergens. Another example is for screening of libraries (DNA.antibodies, etc) for different reactivities.

Brief description of drawings

Fig. 1 is a perspective view of an embodiment of a device according to the present invention.

Fig. 2 is a sectional side view of the device in Fig 1;

Fig. 3 is an exploded view corresponding to the side view in Fig. 2.

Detailed description of the invention

As shown in Fig. 1 the device comprises an upper housing part 1 and a lower housing part 2 of material which is inert with respect to the sample an any reagents used in the assays to be conducted with the device, e.g. polystyrene or polypropylene. The upper housing part 1 has a sample well aperture 3 (here conical) and a detection window 4.

The lower housing part 2 has mounted therein a membrane strip 6 of biboulous material (i.e. a porous material susceptible to traversal of an aqueous medium due to capillary action), e.g. nitro-cellulose on a polyester backing. Near the upstream end of the strip 6 (to the left in the figures), a filter piece 7, containing diffusely movable detection reagent (labelled second binding reagent), is placed on the strip. Such a detection reagent may, for example, be a conjugate between a label particle and a reactant capable of binding to the analyte. Further downstream, and placed below and within the detection window 4, there is a multi-spot reaction zone 8 on the strip which contains several capturing agents or reactants immobilised in a specific pattern on the strip. The capturing agents are capable of binding to the analytes to be tested for. The reaction zone 8 (Fig. 2-3) may be smaller or larger than in the shown figures and may contain 5-1000 capturing agents, preferably 10-100 capturing agents. Importantly, capturing agents having cross-reacting analytes will optionally not be arranged in the same lane, i.e. not in the same flow line of liquid.

The upper housing part 1 contains at the upstream end of the membrane strip 6, a pad 11 of liquid absorbing material intended to serve as a container for flow liquid, or buffer. The opening 3 in the housing part 1 is intended for introducing sample to the membrane 6. In the illustrated case, a filter element 12 (which optionally may consist of two or more filters), is provided below the opening 3 for assays where the sample liquid needs to be filtered, e.g. when the sample is whole blood and blood cells are to be separated off. The buffer pad 11 thus forms a buffer liquid container, below referred to as buffer pad, and the room defined by the sample opening 3 and the filter element 12 forms a sample well, or sample container.

Optionally, a pull-out film 5 is present the purpose of which will be described further below. At the downstream end of the membrane strip 6, a wicking element 13 is placed, here in the form of a pad of absorbent material, such as cellulose, the purpose of which is to assist in maintaining a capillary flow of assay liquids through the membrane strip 6.

An assay for analytes in a sample may be performed with the device described above as follows.

The device is usually provided ready for use with the buffer pad 11 soaked with buffer solution (flow liquid), with the detection reagent pre-deposited in the filter 7, and with the

respective appropriate capture agents and calibration agents immobilised in a specific pattern of spots in the reaction (or detection) zone 8. This offers a possibility to optimally position the calibration spots among the other spots.
The function of the calibration spots is as a positive control and/or internal calibrator.

If the analyte to be tested for is, say, an antigen, the detection reagent in the filter 7 may, for example, be an antibody to the antigen coupled to a fluorogen-labelled particle, the immobilised capturing agents in the multi-spot reaction zone 8 may be antibodies, and the calibrator agent may be the analyte or an analyte analogue.

A predetermined amount of sample is added through the opening 3 in the housing part 1. All the necessary assay liquids, i.e. in this case sample liquid and buffer liquid, are then present in the device, the pull-out film 5, however, effectively preventing contact between the respective liquids and the membrane strip 6. The assay is then started by the operator removing the pull-out film 5 to thereby put the membrane strip 6 in simultaneous liquid receiving contact with the buffer pad 11 and the sample liquid in the sample well 3. If the pull-out film is not present, the assay will start directly following sample addition.

Buffer liquid from the pad 11 will now penetrate into the membrane strip 6 via the far upstream end part thereof which is in direct contact with the pad 11 (see Fig. 3) and be transported downstream the membrane strip 6 by capillary force. Simultaneously, sample liquid directly followed by a (first) flow pulse of buffer liquid. However, the detection reagent filter 7 and a major part of the buffer pad 11 are separated from the membrane strip 6 by the flow barrier film 10. Buffer liquid that has been transported into the membrane strip 6 will penetrate into and be transported through the filter 7 and bring the detection reagent deposited therein with it, thereby forming a detection reagent flow pulse. This detection reagent flow pulse will follow in sequence after the sample flow and the buffer flow pulse. Buffer that is transported in the membrane strip 6 after the detection reagent has been removed from the filter 7 will form a second buffer flow pulse following after the detection reagent flow pulse.

The above-mentioned different liquid flows will be transported along the membrane strip 6 in the indicated sequence, i.e. sample flow, first buffer flow, detection reagent flow, and second buffer flow, and will eventually reach the multi-spot reaction zone 8. In the reaction zone 8,

analytes present in the sample will be captured by the reagents immobilised in the specific spot pattern in the membrane. The analyte/capture reagent complexes formed will be washed by the following first buffer flow, and the flow of detection reagent will form detectable reagent/analyte complexes in the reaction zone. The latter will finally be washed by the second buffer flow. In the calibration spots, the predetermined amount of analyte therein will react with the detection reagent in the detection reagent flow to form a detectable detection reagent/analyte complex. By measuring the signal intensity from the detection reagent captured in the reaction zone and correlate it with that obtained in the calibration spot(s), the amount of analyte in the sample may be determined.

In the reaction (or detection) zone 8 described above, several reactants capable of specifically binding to analytes are immobilised in a specific spot pattern (by covalent binding, via physical adsorption, via biospecific affinity, via immobilised particles to which the reactant is covalently bound, etc.). However, instead an agent capable of reacting with the reactant may be immobilised in the membrane, and the reactant may then be added together with the sample, or be pre-deposited in the membrane in an area or zone upstream of the reaction zone. Such an immobilised agent may be one member of a specific binding pair (sbp) and the reactant is then coupled or conjugated to the other member of the spb. Exemplary specific binding pairs include immunological binding pairs, such as antigen-antibody and hapten-antibody, biotin-avidin or -streptavidin, lectin-sugar, hormone-hormone receptor, nucleic acid duplex. For example, the reaction zone may have streptavidin immobilised therein and the capture reactant for the analyte may be biotinylated.

Similarly, the calibration spot(s) may contain a binder for the calibrator substance rather than the calibrator substance *per se*. The binder is usually a member of a specific binding pair, such as one of those mentioned above, whereas the other member of the specific binding pair is coupled or conjugated to the calibrator substance, which may in turn be added with the sample or pre-deposited upstream of the calibrator zone. Streptavidin, for example, may be immobilised in the calibrator zone while the calibrator substance is biotinylated.

For further details on assay devices of the type contemplated herein, and particularly regarding flow matrixes, sequential assays, calibrator systems and detection reagents, it may

be referred to our published PCT applications WO 99/36776, WO 99/36777 and WO 99/36780, for example.

Analytes to be determined using the present device are readily apparent to the skilled person. Usually, however, the analyte is a biospecific affinity reactant, e.g. an antibody or other protein, hapten, nucleic acid or polynucleotide, such as a DNA sequence. In the latter case the reaction zone may contain streptavidin and the DNA sequence to which the analyte sequence is to hybridise to may be biotinylated.

The present device permits convenient pre-treatment of the sample before starting the assay.

The present device may also be adapted for performing assays of the type described in our published PCT application WO 99/60402 where the flow matrix contains a chromatographic separation zone upstream of the reaction (detection) zone to separate sample components which would otherwise disturb or influence the determination of the analyte.

Claims

1. A device for determining analytes in an aqueous sample comprising: an elongate flow matrix (6) allowing lateral transport of fluid therethrough, wherein said matrix comprises a sample application zone (3) and downstream thereof, a detection zone (8) having immobilised capture agents capable of directly or indirectly binding to said analytes, wherein said analytes are detected by allowing a labelled second binding agent to bind directly or indirectly to the analytes, **characterised in** that A) the immobilised capture agents are distributed in the detection zone (8) as a plurality of small spots, thereby permitting multi-analyte and/or multi-specificity detection, and B) the capture agents are anchored to the matrix via immobilised particles, and C) the number of spots per flow matrix is more than 10, and D) wherein some of the spots functions as positive control(s) and/or internal calibrator(s).

2. A device according to claim 1, wherein the spots are smaller than 1 mm in diameter, preferably smaller than 0,5 mm in diameter.

3. A device according to claim 1 or 2, wherein the spots are arranged in a pattern that allows for detection of cross reactive analytes or specificities, i.e. cross reacting analytes are not arranged in the same flow line of liquid.

4. A device according to any of the above claims, wherein the flow matrix is a porous membrane.

5. A device according to any of the above claims 1-3, wherein the matrix is a strip of solid material.

6. A device according to any of the above claims 1-5, wherein the capture agents are antibodies or an immunoactive fragment thereof.

7. A device according to any of the above claims 1-5, wherein the capture agents are allergens or an immunoactive fragment thereof.

8. A device according to any of the above claims 1-5, wherein the capture agents are autoantigens or an immunoactive fragment thereof.

9. A device according to any of the above claims 1-5, wherein the capture agents are DNA/RNA, , preferably single stranded nucleic acids or aptameres, or DNA/RNA like structures.

10. A device according to any one of the above claims, wherein the sample is whole blood, serum, plasma, saliva or urine.

11. A device according to any of the above claims, wherein the label is a fluorophore or a chromophore.

12. Use of the device according to one or more of the above claims 1-11 for screening of unknown specificities.

13. Use of the device according to one or more of the above claims 1-11 for screening of specific immunoglobulins.

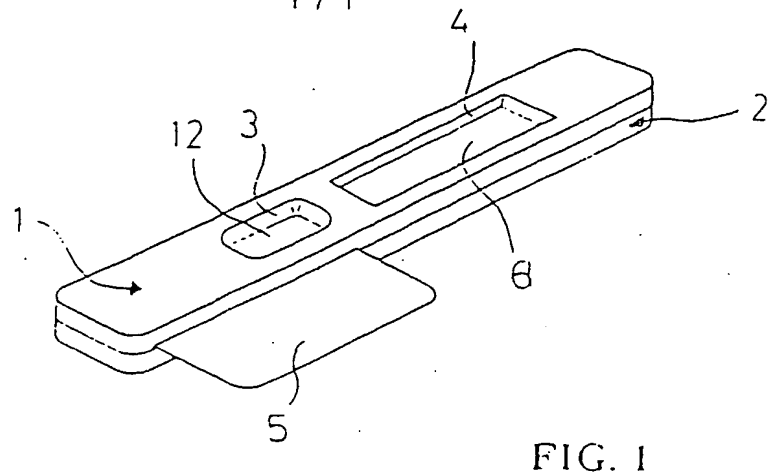


FIG. 1

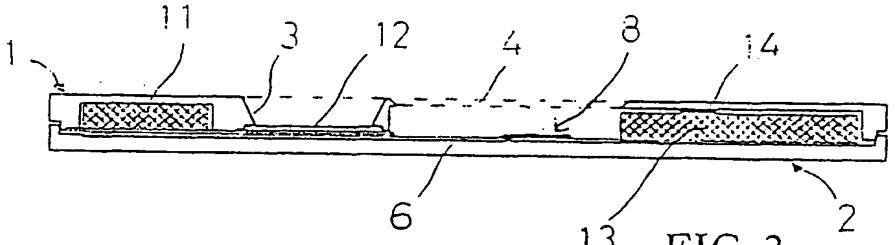


FIG. 2

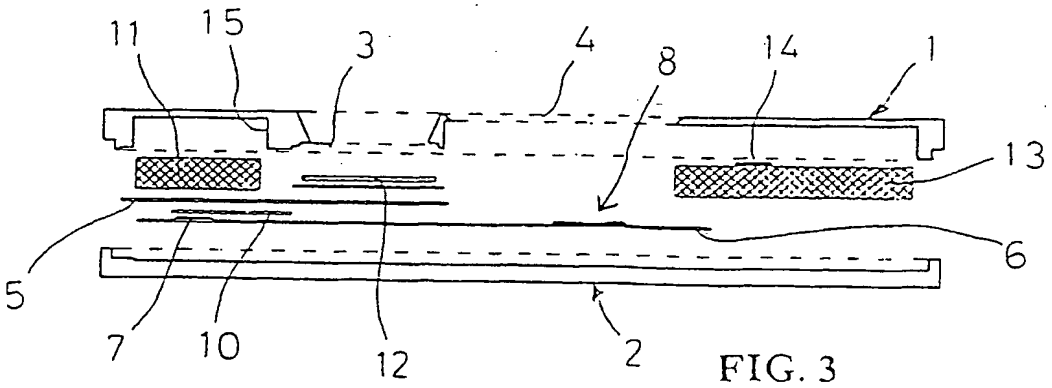


FIG. 3

INTERNATIONAL SEARCH REPORT

International application No.
PCT/SE 02/01671

A. CLASSIFICATION OF SUBJECT MATTER

IPC7: G01N 33/543, C12Q 1/68
According to International Patent Classification (IPC) or to both national classification and IPC

B. FIELDS SEARCHED

Minimum documentation searched (classification system followed by classification symbols)

IPC7: G01N, C12Q

Documentation searched other than minimum documentation to the extent that such documents are included in the fields searched

SE,DK,FI,NO classes as above

Electronic data base consulted during the international search (name of data base and, where practicable, search terms used)

EPO-INTERNAL, BIOSIS, MEDLINE

C. DOCUMENTS CONSIDERED TO BE RELEVANT

Category*	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
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[X] Further documents are listed in the continuation of Box C. [X] See patent family annex.

* Special categories of cited documents:
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Date of the actual completion of the international search	Date of mailing of the international search report
19 December 2002	09 -01- 2003
Name and mailing address of the ISA/ Swedish Patent Office Box 5055, S-102 42 STOCKHOLM Facsimile No. +46 8 666 02 86	Authorized officer CARL-OLOF GUSTAFSSON/BS Telephone No. +46 8 782 25 00

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INTERNATIONAL SEARCH REPORT

International application No.
PCT/SE 02/01671

C (Continuation). DOCUMENTS CONSIDERED TO BE RELEVANT

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INTERNATIONAL SEARCH REPORT

Information on patent family members

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